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Philippe Juneau, Alexandre Barnett, Vona Méléder, Christine Dupuy, Johann Lavaud. Combined effect of high light and high salinity on the regulation of photosynthesis in three diatom species belonging to the main growth forms of intertidal flat inhabiting microphytobenthos. Journal of Experimental Marine Biology and Ecology, 2015, 463, pp.95-104. 10.1016/j.jembe.2014.11.003 . hal-01095784

HAL Id: hal-01095784
https://hal.science/hal-01095784

Submitted on 16 Dec 2014

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Combined effect of high light and high salinity on the regulation of photosynthesis in three diatom species belonging to the main growth forms of intertidal flat inhabiting microphytobenthos

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Abstract

The strong biological production of estuarine intertidal flats is mainly supported by benthic diatoms in temperate areas. Their photosynthetic productivity is largely driven by changes in light intensity and temperature at the surface of sediment flats during emersion. The impact of an increase in salinity of the upper-layer sediment pore-water during emersion, which is often coupled with high light (HL), has been less studied. Furthermore, benthic diatoms show several growth forms which inhabit specific sediment types where the pore-water salinity can differentially vary due to the degree of cohesion of sediment grains. So far, no study explored if the main growth forms of benthic diatoms (i.e. epipelon, epipsammon and tychoplankton) show different photophysiological response to a combine high salinity-HL stress. Based on field monitoring, we compared the photophysiology (photosynthetic efficiency and photoprotection) of three representatives of the main growth forms during a short high salinity coupled with a moderate HL stress and stable optimal temperature, i.e. experimental conditions reproducing Spring environmental conditions in intertidal flats by the Atlantic French coast. Our results show that all growth forms reacted to HL exposure alone, as expected. While the epipelon representative was relatively insensitive to high salinity alone and combined with HL, the tychoplankton representative was highly sensitive to both, and the epipsammon representative was sensitive mainly to the stress combination. These specific responses fitted well with i) their natural habitat (i.e. more or less cohesive sediment) for which light climate and changes in salinity are different, ii) their growth form (i.e. motile, immotile or amphibious) which determines their probability to be confronted to a combined high salinity-HL stress. Hence, the negative effect of high salinity on photosynthetic efficiency of benthic diatoms

appears to be mostly restricted to epipsammon and tychoplankton, and in field conditions, its effect probably remains negligible compared to HL stress.

Keywords: diatom / intertidal flat / microphytobenthos / photoprotection / photosynthesis / salinity.

List of abbreviations: Chl *a*, chlorophyll *a*; DD, diadinoxathin; DES, de-epoxidation state of diadinoxanthin to diatoxanthin; DT, diatoxanthin; E, light intensity; ETR, electron transport rate; HL, high light; LL, low light; MPB, microphytobenthos; NPQ, non-photochemical quenching of chlorophyll fluorescence; PSII, photosystem II; RLC, rapid light curve; XC, xanthophyll cycle

1. Introduction

Estuarine intertidal flats belong to the most productive ecosystems on Earth (MacIntyre et al., 1996; Underwood and Kromkamp, 1999) and they have a central role in structuring the food-web of coastal areas (Kromkamp and Forster, 2006). A large part of the strong productivity of intertidal flats is due to the microphytobenthos (MPB) (Admiraal, 1984; MacIntyre et al., 1996; Underwood and Kromkamp, 1999) which in temperate seas is mainly dominated by benthic diatoms (Méléder et al., 2007; Ribeiro et al., 2013). Benthic and planktonic diatoms are essential primary producers which contribute to about 40% of the marine primary production; they also play a major role in the silica and nitrogen biogeochemical cycles (Armbrust, 2009). The MPB diatoms constitute the bulk of the diatom diversity (Kooistra et al., 2007). They can be divided in three main growth forms which mainly differ in their life in the sediment (Kooistra et al., 2007; Ribeiro et al., 2013): i) the epipelon comprises motile species free-living in between sediment particles (Herlory et al., 2004), ii) the epipsammon which lives attached to sediment particles, and iii) the tychoplankton which presumably have an amphibious life style (i.e. both sediment and water column) (e.g. Sabbe et al., 2010). Epipelon and epipsammon growth forms show distinct distribution among intertidal habitats characterised by different types of sediment (Sabbe, 1993; Méléder et al., 2007; Ribeiro et al., 2013). Epipelon dominates cohesive muddy sediments (> 90% of MPB; Haubois et al., 2005), while epipsammon dominates less cohesive sandy sediments (> 95% of MPB; Méléder et al., 2007). Because of different habitats, epipelon and epipsammon have evolved different ways of coping with their intertidal environment. Epipelon displays vertical ‘migration’ following endogenous tidal/dial rhythms and environmental stimuli (Saburova and Polikarpov, 2003; Consalvey et al., 2004; Coelho et al., 2011): typically, during daylight emersion, epipelic

diatoms move to the sediment surface and form a dense biofilm, while before immersion they migrate downward. Epipsammon lives more or less firmly attached (stalked or adnate forms) to individual sand grain including some species able to exert micro-movements within the sphere of grains. Tychoplankton (which is sometimes considered as resuspended epipelon and/or epipsammon during immersion; MacIntyre et al., 1996) can live either as part of MPB or of phytoplankton, depending on the hydrodynamics (Koh et al., 2006); it can contribute to up to one third of phytoplankton (Guarini et al., 2004; Brito et al., 2012).

Environmental cues can rapidly vary to an extreme in intertidal flats (Admiraal, 1984; Paterson and Hagerthey, 2001) and impair the photosynthetic productivity of MPB diatoms (i.e. photoinhibition) (Blanchard et al., 2004; Serôdio et al., 2008). In order to prevent such situation, benthic diatoms have evolved diverse responses that can be distinguished in two main types: behaviour and physiology. Only epipelon can escape from a combination of sometimes harsh environmental conditions at the sediment surface by 'migrating' downward to the most optimal conditions (i.e. the so-called 'behavioural photoprotection'; (Admiraal, 1984; Kromkamp et al., 1998; Consalvey et al., 2004; Serôdio et al., 2006), especially as regards to salinity (Sauer et al., 2002). In contrast, all growth forms use physiological processes for the fast regulation of photochemistry (i.e. 'physiological photoprotection'; (Lavaud, 2007; Goss and Jakob, 2010; Depauw et al., 2012; Lepetit et al., 2012). In diatoms, two physiological processes are important in field situation (Brunet and Lavaud, 2010; Lavaud and Goss, 2014): i) the non-photochemical quenching of chlorophyll (Chl) fluorescence (NPQ) (Depauw et al., 2012; Lepetit et al., 2012; Lavaud and Goss, 2014), and ii) the partly related light-dependent conversion of diadinoxanthin (DD) to diatoxanthin (DT) by the DD de-epoxidase (i.e. the 'xanthophyll cycle', XC) (Brunet and Lavaud, 2010; Goss and Jakob, 2010). In benthic

diatoms, NPQ and XC have been scarcely studied *in situ*: it varies with the diurnal and tidal cycles, season, latitude (Serôdio et al., 2005; van Leeuwe et al., 2009; Chevalier et al., 2010; Serôdio et al., 2012), and with the position of diatom cells within the sediment and along the intertidal elevation gradient (Jesus et al., 2009; Cartaxana et al., 2011). The respective importance of behavioural and physiological responses in epipelon has received a major interest (Mouget et al., 2008; van Leeuwe et al., 2009; Perkins et al., 2010b; Cartaxana et al., 2011; Serôdio et al., 2012). These studies have shown that although motility is essential for an optimal response to the changes in environmental conditions, NPQ and XC remain important features, and even compensate for migration under conditions where motility is limited, to finely tune photosynthetic efficiency. Also, a recent analysis of NPQ and XC abilities among the growth forms of MPB diatoms has revealed a clear relationship between growth form and capacity for physiological photoprotection (Barnett et al., 2014), i.e. while epipsammon shows the highest NPQ and XC capacity, epipelon and tychoplankton shows the lowest ones, reflecting their respective motility and adaptation to a low light (LL) environment (i.e. tychoplankton is either buried in sediment or resuspended in a turbid water column; Roncarati et al., 2008).

Changes in light intensity and temperature are often considered as the two major forcings of the photosynthetic productivity of MPB diatoms (Guarini et al., 2006). Surprisingly, changes in salinity have been less studied in benthic diatoms, while in planktonic diatoms it is known to induce modification of community species diversity (Thessen et al., 2005; Dijkman and Kromkamp, 2006; Muylaert et al., 2009; Petrou et al., 2011), and of growth and photosynthesis (Thessen et al., 2005; Dijkman and Kromkamp, 2006; Petrou et al., 2011). Salinity often co-varies with other environmental gradients like light and temperature in the case of high

126 salinities (due to pore-water evaporation in the upper-layer of the sediment) and with nutrient
127 concentrations in the case of low salinities (due to the discharge of estuarine rivers) (Admiraal
128 and Peletier, 1980; Underwood and Provot, 2000; Thornton et al., 2002). Although early works
129 stated that MPB diatoms are highly tolerant to a wide range of salinity changes (Williams,
130 1964; Admiraal, 1977; Admiraal and Peletier, 1980), further studies have shown that salinity
131 changes, often combined with high light (HL), impairs the growth from a salinity of 40 and
132 above (Natana Murugaraj and Jeyachandran, 2007; Scholz and Liebezeit, 2012), it reduces the
133 photosynthetic performance (Roncarati et al., 2008; Le Rouzic, 2012) via (photo-)oxidative
134 stress (Rijstenbil, 2003, 2005; Roncarati et al., 2008), and it can modify the motility of epipelic
135 diatoms in the sediment (Sauer et al., 2002) via changes in the excretion of
136 exopolysaccharides (Apoya-Horton et al., 2006). Furthermore, although the different growth
137 forms of MPB diatoms pertain to habitats in which the salinity can differentially vary due to
138 the degree of cohesion of sediment (Paterson and Hagerthey, 2001), to our knowledge, no
139 study explored if they show different photophysiological response to a combine high salinity-
140 HL stress and if it correlates to their habitat-associated growth form. The objectives of the
141 present study were therefore to determine i) if a higher salinity can increase the negative effect
142 of HL on the photosynthetic efficiency, ii) if three representatives belonging to each of the
143 growth forms of MPB diatoms react differently to a combined high salinity-HL stress.
144
145 *2. Materials and methods*
146 *2.1. Sediment grain size, pore-water salinity, temperature and MPB biomass of sediment*
147 Parameters were measured at different seasons and for two sites of the Atlantic French coast:
148 the bay of Brouage and the bay of Bourgneuf; see Haubois et al. (2005) and Méléder et al.

149 (2007) for a respective characterization of the two sampling sites (see Table 1 and Figure 1 for
150 all details). Sediment grain size was determined with a laser granulometer (Mastersizer 2000,
151 Malvern Instruments, UK) as previously described (Méléder et al., 2007). The mud fraction
152 (grain size < 63 µm) of each sample was determined using the software Gradistat (Blott and
153 Pye, 2001). Sediment samples were centrifuged for 10 min at 3500 g and salinity was
154 measured on the supernatant with a sensor TetraCon325 (WTW, Weilhem, Germany). The
155 temperature at the sediment surface was measured every 30 s with a universal data logger
156 (ULM-500, Walz Effeltrich Germany) equipped with a plane temperature sensor (accessory of
157 the ULM-500). The sediment content of chlorophyll *a* (µg Chl *a*. g dry sediment^{-1}) was used as
158 a proxy for MPB biomass. Chl *a* was extracted and measured as previously described (Herlory
159 et al., 2004): spectrofluorimetric measurement (Turner TD-700 fluorometer) was performed on
160 supernatant of sediment samples after lyophilisation, extraction (90% acetone, 12 h, 4°C, in the
161 dark, continuous shaking) and centrifugation 10 min at 4000 g.
162
163 *2.2. Diatom culture conditions*
164 Three species belonging to the three main growth forms of MPB diatoms were used: 1)
165 Epipelon, *Navicula phyllepta* (Culture Collection Yerseke-The Netherlands CCY9804,
166 isolated in the Westerschelde estuary, North sea, The Netherlands); 2) Epipsammon, *Biremis*
167 *lucens* (Nantes Culture Collection-France NCC360, isolated in the bay of Bourgneuf,
168 Atlantic,France; 3) Tychoplankton, *Plagiogrammopsis vanheurckii* (NCC186-2, isolated in the
169 bay of Bourgneuf). Cultures were grown in batch sterile artificial seawater F/2 medium
170 completed with Tropic Marin artificial sea salt (Dr. Biener GmbH, Germany) at a salinity of
171 33, and enriched with $NaHCO_3$ (80 mg L^{-1} final concentration). Temperature was 20°C and

light was 60 µmol photons m^{-2} s^{-1} (white fluorescent tubes L58W/840, OSRAM, Germany) with a 16 h:8 h light:dark photoperiod. When cultures reached exponential phase, cells were harvested by gentle centrifugation (5 min, 4000 g), resuspended to a concentration of 6 ± 1 mg Chl *a* mL^{-1}. For this purpose, Chl *a* concentration was determined according to the Jeffrey and Humphrey (1975) spectrophotometric method. Diatom suspensions were continuously stirred under the growth conditions for at least 1 h before the high light (HL) and salinity treatments.

2.3. High light (HL) and salinity treatments

Diatom cells were exposed for 1 h to a range of increasing salinities (33, 37, 41 and 45) under the growth light intensity and under HL intensity (10x the growth light intensity, 600 µmol photons m^{-2} s^{-1}) at 20°C. During each treatment, cells were stirred to prevent settling. Each condition was measured in triplicate. The temperature, light and salinity values/ranges were chosen according to the *in situ* measurements (see Table 1 and Figure 1) in order to reproduce the environment experienced by MPB diatoms in Spring (see the Results section, paragraph 3.1). Increased salinity was obtained by implementing the sterile artificial seawater F/2 medium with increasing amounts of Tropic Marin artificial sea salt (Dr. Biener GmbH, Germany). HL was provided by white fluorescent tubes (FQ 54W/865 LO, OSRAM, Germany).

2.4. Pigment analyses

At the end of each salinity and light treatments, 1 mL of diatom suspension was filtered on a membrane filter (Membrane Isopore Polycarbonate 1.2-µm RTTP filter, 25 mm diameter, Merck Millipore, Ireland), quickly frozen in liquid nitrogen and stored at -80°c until further

analysis. Pigment extraction and determination of pigment content were performed as previously described (Barnett et al., 2014). Chl *a*.cell^{-1} was calculated by counting the number of cells microscopically with a Malassez's counting chamber. The de-epoxidation state (DES in %) was calculated as DES = [(DT / DD + DT) x 100], where DD is the diadinoxanthin, the epoxidized form, and DT is the diatoxanthin, the de-epoxidized form.

2.5. Chl fluorescence yield and rapid light curves (RLCs)

For a complete overview of the definition, measurement and calculation of the fluorescence levels and of the photophysiological parameters, see Table 2. Chl fluorescence yield was monitored with a Diving-PAM fluorometer (Walz, Germany) on a 2.5 mL stirred and 20°C controlled diatom suspension (see Lavaud et al., 2004). Before measurement, the cells were dark-adapted for 15 min, and a saturating pulse (3600 µmol photons m^{-2} s^{-1}, duration 0.4 ms) was fired to measure F_0, F_m and F_v/F_m. For RLCs (Perkins et al., 2010a), the diatom suspension was exposed to 8 successive increasing intensities (29-1038 µmol photons.m^{-2}.s^{-1}) of 60 s each. At the end of each RLC-light step exposure, F_m' was measured. RLCs allow constructing rETR *vs*. E curves; from the fitted rETR-E curves (Eilers and Peeters, 1988), $rETR_m$, α, E_k can be extracted.

3. Results

3.1. Pore-water salinity and MPB biomass in different sediments of the French Atlantic coast

The changes in pore-water salinity in the upper layer (first 1 cm) was measured over different seasons at two sites of the French Atlantic coast characterised by two sediment types: 1) a site with 95% of cohesive muddy sediment which is known to be dominated by a community of

218 epipelic diatoms, and especially *Navicula phyllepta*, throughout the year (Haubois et al.,
219 2005); 2) a site with a mix of muddy and sandy (thus less cohesive) sediment which is known
220 to be dominated by a community of epipsammic diatoms where *Biremis lucens* and
221 *Plagiogrammopsis vanheurckii* are typical (Méléder et al., 2007). Overall, pore-water salinity
222 varied between 29 and 48 during the 3 h emersion period (Table 2). As expected, variations
223 over an emersion were higher in summer than in winter, and over seasons in cohesive than in
224 less cohesive sediment with an overall minimum and maximum variation during an emersion
225 of 2.3 and 8.3, respectively.
226 The changes in pore-water salinity were further deciphered at the two sites during the course of
227 an emersion in Spring at two depths in the upper layer of sediments (Fig. 1). Large changes in
228 pore-water salinity occurred within only 1.5 h: mean Δ 5.1 and Δ 3.5 for the muddy and the
229 muddy-sandy sediment, respectively. Nevertheless, these changes were mainly (muddy
230 sediment, Fig. 1A) and even exclusively (muddy-sandy sediment, Fig. 1B) observed in the first
231 0.5 cm where most of the MPB biomass was present (Fig. 1C). In the deeper sediment layer (-
232 0.5-1 cm), a high equivalent MPB biomass (40.5 ± 3.5 and 48.5 ± 5.3 µg Chl *a* g sediment^{-1} in
233 muddy and muddy-sandy sediment, respectively) was observed and the pore-water salinity was
234 close to 33: 34.0 ± 0.7 in mud and 33.0 ± 1.3 in muddy sand.
235
236 *3.2. Photophysiological response of* Navicula phyllepta *(epipelon),* Biremis lucens
237 *(epipsammon) and* Plagiogrammopsis vanheurckii *(tychoplankton) to a combined high*
238 *salinity-HL stress*
239 The PSII activity of the three species was assessed by measuring F_v/F_m and ΦPSII, as well as
240 the RLCs photophysiological parameters (α, $rETR_m$ and E_k) (see Table 2). While in LL-

241 acclimated cells F_v/F_m and ΦPSII did not change significantly with salinity (Tables 3 and 5),
242 HL treatment induced a significant mean decrease in F_v/F_m (*N. phyllepta*, -8.3 ± 1.0 % < *B.*
243 *lucens*, -11.3 ± 1.7 % < *P. vanheurckii*, -37.3 ± 1.7 %) and in ΦPSII (*N. phyllepta*, -6.3 ± 1.5 %
244 < *B. lucens*, -10.0 ± 3.3 %) independent of salinity (Tables 3 and 5). Only in *P. vanheurckii*
245 ΦPSII changes were salinity-dependent, i.e. -11.5 ± 3.5 % from 33-37 and -28.5 ± 1.5 % for
246 41-45 (Table 3). The RLCs photophysiological parameters (α, $rETR_m$ and E_k, see Table 2)
247 changed differently depending on the species and salinity/light treatments. *N. phyllepta* cells
248 were not significantly affected either by changes in salinity nor by the HL treatment (Fig. 2
249 and Table 5). In *B. lucens* LL acclimated cells, E_k and $rETR_m$ were significantly affected by
250 salinity (Fig. 2 and Table 5) and, as expected, α and E_k were significantly lower and higher,
251 respectively, after HL treatment (Table 5) although only for a limited range of salinities: up to
252 41 for α and up to 37 for E_k. In HL cells, further salinity raising induced an increase in α
253 (+25.5 ± 3.5 %) and a subsequent decrease in E_k (-39 ± 3 %) (Fig. 2). It was in *P. vanheurckii*
254 α, $rETR_m$ and E_k showed the most pronounced changes with salinity and HL treatment (Fig. 2
255 and Table 5). α decreased in both LL acclimated cells (-23 ± 14 %) and after HL treatment (-39
256 ± 5 %, especially from a salinity of 33 to 37). $rETR_m$ followed an opposite trend, and
257 significantly increased with salinity by a mean factor of up to 1.60 ± 0.13, and independent of
258 the light treatment (Table 5). As a consequence, E_k significantly increased together with
259 salinity for both light treatments (x 1.96 ± 0.3 and x 1.53 ± 0.3 for HL and LL cells,
260 respectively).
261 The photoprotective response of the species was assessed by measuring NPQ and the XC
262 components and operation (Tables 3 and 4). NPQ_m of LL acclimated cells (Table 3) was on

263 average 0.224 ± 0.135 (*N. phyllepta*), 0.332 ± 0.018 (*B. lucens*) and 0.645 ± 0.105 (*P.*
264 *vanheurckii*) and, as expected, it significantly increased during the HL treatment (Tables 3 and
265 5) as follows: *P. vanheurckii* (x 1.6 ± 0.3 for all salinities) < *N. phyllepta* (x 1.8 ± 0.3 for all
266 salinities except 45, x 2.5) < *B. lucens* (x 2.2 ± 0.4 for all salinities). Only in *P. vanheurckii* LL
267 acclimated cells, NPQ_m significantly decreased by -30% from a salinity of 33 to 45 (Tables 3
268 and 5). DES was much higher in LL acclimated cells of *P. vanheurckii* (40 ± 4 %) than in *N.*
269 *phyllepta* (12 ± 3 %) and *B. lucens* (7 ± 1 %) (Fig. 3). During the HL treatment, and
270 independent of salinity, DES significantly increased to a different extent (*P. vanheurckii*, 58 ±
271 2 % > *B. lucens*, 39 ± 3 % > *N. phyllepta*, 31 ± 2 %) (Fig. 3 and Table 5). Interestingly, in *N.*
272 *phyllepta*, while diatoxanthin (DT) significantly increased during HL (i.e. due to
273 diadinoxanthin, DD, de-epoxidation), DD did not similarly decreased (Tables 4 and 5), as it
274 would have been expected, arguing for a *de novo* synthesis of DD during HL. Out from DD
275 and DT, there was no additional significant pigment changes in the three species whatever the
276 treatment except a significant HL-salinity independent decrease in Chl *a*.$cell^{-1}$ (-20.3 ± 4.5 %)
277 in *N. phyllepta*.
278 The higher sensitivity of *P. vanheurckii* to salinity alone was further illustrated by the fact that
279 the combination with the HL treatment did not significantly change some of the
280 photophysiological parameters (α, $rETR_m$, E_k, NPQ_m) in contrast to *N. phyllepta* and *B. lucens*
281 (Table 5).
282
283 *4. Discussion*

4.1. Pore-water salinity changes in Atlantic French coast intertidal flats and their potential effects on intertidal MPB diatoms

In the field, the upper layer sediment pore-water salinity can highly (Δ 5) and rapidly (within 1.5 h) increase, and reach values as high as 48 in Summer (values up to 55-60 were even reported elsewhere, Roncarati et al., 2008; Serôdio et al., 2008). Nevertheless, high salinity events are not restricted to hot sunny days and also occur at moderate temperature (16-20°C), as shown here in Spring, due to wind-driven desiccation (Williams, 1964; Sauer et al., 2002) in the first 0.5 cm of the sediment (where the bulk of the MPB biomass inhabits). Changes in the sediment pore-water salinity depend on the sediment cohesion with higher values and amplitude in cohesive sediment probably due to the trapping (and subsequent higher evaporation) of pore-water at the surface (Paterson and Hagerthey, 2001; Sauer et al., 2002). Therefore, although temperature may be optimal (20-25 °C, Blanchard et al., 1997; Scholz and Liebezeit, 2012), HL and high salinity conditions may occur in the sediment upper layer during Spring-early Summer emersion by the Atlantic French coast. These conditions may differentially impair the photosynthetic efficiency of the main growth forms of MPB as regards to the sediment cohesion of their respective habitat or their amphibious life. The HL-high salinity combination has been rarely studied before (Rijstenbil, 2003, 2005; Roncarati et al., 2008). Most previous works focused on low salinity stress combined to nutrient gradient due to estuarine rivers discharge (Admiraal and Peletier, 1980; Underwood and Provot, 2000; Thornton et al., 2002) and on the long-term effect of salinity changes (most often measured by specific growth, Williams, 1964; Jackson et al., 1992; Underwood and Provot, 2000; Natana Murugaraj and Jeyachandran, 2007; Scholz and Liebezeit, 2012), instead of effects (including

306 short-term exposure) on the photosynthetic efficiency (Admiraal, 1977; Admiraal and Peletier,
307 1980; Roncarati et al., 2008).
308
309 *4.2. Differential photosynthetic and photoprotective response to a combined high-salinity-HL*
310 *stress in diatoms representative of the main growth forms of intertidal MPB*
311 The photosynthesis of the three examined species responded differently to the combination of
312 HL and salinity stress. In our conditions, *N. phyllepta*, the photosynthetic efficiency was not
313 largely impaired neither by high salinity nor HL alone or both in combination (i.e. changes in
314 PSII and RLCs photophysiological parameters were not > 10 % on average). Noticeably, HL
315 induced a decrease in Chl *a*. cell^{-1} that was not observed in the two other species. It shows the
316 ability to lower the overexcitation of the whole photosynthetic machinery under HL stress
317 (Brunet et al., 2011). In contrast, in *B. lucens*, the photosynthetic electron transport rate
318 ($rETR_m$) was slightly but significantly affected by high salinity alone. Additionally, the
319 photoacclimatory-coupled decrease in α and increase in E_k, that illustrates the decrease of the
320 excitation pressure on PSII (Perkins et al., 2006; Cruz and Serôdio, 2008; Lefebvre et al.,
321 2011), were abolished by higher salinities (from 37 on). Nevertheless, in our moderately
322 stressful conditions, it did not largely impaired PSII activity (decrease in F_v/F_m and ΦPSII at
323 maximum ~11%). The high salinity-dependent inhibition of photoacclimation was not
324 observed in *P. vanheurckii* for which the decrease of the excitation pressure on PSII was
325 obviously a key response in all conditions. While under HL alone, α and E_k modulation was
326 enough, under high salinity alone and combined with HL, the additional increase in $rETR_m$,
327 possibly through a stronger activation of the Calvin cycle enzymes (Nymark et al., 2009), was
328 necessary to cope with the stress. These changes in the photosynthetic efficiency were

329 sufficient for *P. vanheurckii* to cope with salinity stress alone but not when it was it was
330 combined with HL (strong ΦPSII decrease for salinities > 41 on), supporting its HL sensitivity
331 (strong F_v/F_m decrease) due to its adaptation to a LL environment (low α and E_k; see also
332 Barnett et al., 2014).
333 In parallel to modification in PSII-related photophysiological parameters, all species exerted a
334 photoprotective response but to a different extent. In contrast to prolonged high salinity
335 exposure (Rijstenbil, 2005), HL DES increase was independent of salinity in all species.
336 Hence, the de-epoxidase enzyme, responsible for the light-dependent conversion of DD to DT,
337 does not seem to be influenced by a short (1 h) salinity stress. DT is well-known to i) scavenge
338 reactive oxygen species (ROS) and to help preventing the peroxidation of lipids of the
339 thylakoid membrane (Lepetit et al., 2010), ii) participate to NPQ (Goss and Jakob, 2010;
340 Lavaud et al., 2012; Lavaud and Lepetit, 2013). In contrast to the two other species, in *N.*
341 *phyllepta* DD de-epoxidation was accompanied by DD *de novo* synthesis, a way to enhance the
342 capacity to further synthesize DT in case of prolonged stress (Lepetit et al., 2013). Probably
343 due to the shortness of high salinity exposure, there was no significant increase in fucoxanthin
344 and β-carotene, a well-known pigment response to oxidative stress additional to the XC
345 (Dambeck and Sandmann, 2014; Tefler, 2014). As expected, NPQ_m was higher in *B lucens*
346 than in *N. phyllepta*, while it was higher than previously observed in *P. vanheurckii* probably
347 due to i) different growth light conditions which generated a higher DES, and ii) the different
348 way of measuring NPQ (RLCs *vs.* Non-Sequential Light Curves-NSLCs) (Barnett et al., 2014).
349 HL NPQ increased was impacted by salinity depending on the species: while NPQ increase
350 was similar (about 1.6x) for all salinities in *P. vanheurckii*, it was higher (about 2.2x) in *B*
351 *lucens*, and it reached an even higher level (2.5x) for a salinity of 45 in *N. phyllepta*. These

observations clearly illustrate how i) NPQ helped to dissipate the excess of light excitation energy in PSII when the photosynthetic machinery was slowed-down by salinity, ii) *N. phyllepta* appeared to be insensitive to all high salinities lower than 45. Strikingly, in *P. vanheurckii*, NPQ decreased linearly (-0.018 ± 0.001 NPQ unit. salinity unit^{-1}) illustrating the high salinity-dependent NPQ inhibition disregard of the high amount of DT synthesised in this species. Most probably here, NPQ decrease and discrepancy between DES and NPQ might be due to a stronger involvement of DT in the prevention of lipid peroxidation by ROS (Lepetit et al., 2010; Lepetit and Lavaud, 2013).

4.3 Relationship between the response of intertidal MPB diatoms to a combined high salinity-HL stress and their habitat-related growth form

The photophysiological response of *N. phyllepta*, *B. lucens* and *P. vanheurckii* to the combined high salinity-HL conditions fitted well with their respective growth form and original habitat. The relationship between photophysiology and the different growth forms of MPB diatoms to light alone was already documented before (Barnett et al., 2014).

4.3.1. Epipelon

N. phyllepta photochemistry was not affected neither by the high salinity stress alone, nor by the combination of HL and high salinity, illustrating an adaptation to potentially extreme conditions of light and salinity at the surface of cohesive (muddy) sediment. This is in agreement with previous reports on the high tolerance to salinity changes of *Navicula* sp. representatives (Underwood and Provot, 2000; Scholz and Liebezeit, 2012), and to a larger extent of epipelon representatives (Williams, 1964; Admiraal, 1977; Admiraal and Peletier,

1980; Clavero et al., 2000). In response to light stress, epipelic diatoms use both vertical motility in the sediment and physiology (Mouget et al., 2008; van Leeuwe et al., 2009; Perkins et al., 2010b; Cartaxana et al., 2011; Serôdio et al., 2012; Barnett et al., 2014). Although in our experiments motility was abolished, the photophysiological response of *N. phyllepta* confirms the likeliness of an equivalent balance between motility and physiology to respond to salinity stress. Surprisingly, *N. phyllepta* did not deploy a strong photophysiological response pointing out to other intra-cellular means that explain its relative insensitivity to high salinity (at least up to 45). For instance, they use proline to adjust their osmotic balance (Natana Murugaraj and Jeyachandran, 2007). Most importantly, cells surround themselves with exopolysaccharides (EPS) to minimize the negative impact of desiccation and high salinity (Sauer et al., 2002) on motility: i) it was shown on a natural assemblage that a shift in salinity from 35 to 45 generated a -30 % migration of the cells at the surface of sediment (Sauer et al., 2002); ii) in controlled laboratory conditions, motility can be even abolished at a salinity of 50 (Apoya-Horton et al., 2006). This phenomenon is based on the decrease of the gliding speed of the cells (Apoya-Horton et al., 2006) and its rapidity (5 s; Apoya-Horton et al., 2006) might be related to intracellular calcium responses (Falciatore et al., 2000; Apoya-Horton et al., 2006). Excretion of EPS during high salinity events allows cell attachment, a prerequisite for cell gliding (Apoya-Horton et al., 2006) that, in field conditions, indeed supports the vertical cell migration to apparently escape extreme salinities. Therefore, the motility response of epipelic diatoms was speculated to be part of an adaptive strategy to respond the sometimes highly changing environment, including light and salinity, at the surface of cohesive sediment, (Admiraal, 1984).

398 *4.3.2. Tychoplankton*

399 Similar to epipelon, tychoplankton movement modalities, when it is buried in sediment at low
400 tide (Roncarati et al., 2008), are strongly influenced by salinity changes (Apoya-Horton et al.,
401 2006). Additionally, high salinity drives the detachment of cells from their substratum, which
402 could be a strategy to avoid longer exposure for this amphibious group (Apoya-Horton et al.,
403 2006). Nevertheless, as reported before (Roncarati et al., 2008) and here, the physiological
404 response to salinity (combined or not with HL) of tychoplankton seems to be more complex
405 than the one of epipelon. They appear highly sensitive to salinities > 35 (Underwood and
406 Provot, 2000) including drastic growth limitation at salinities > 40 (Rijstenbil, 2003; Scholz
407 and Liebezeit, 2012). In our conditions, salinities from 35 on generated a strong
408 photophysiological response in *P. vanheurckii*: its photochemical machinery acclimated just
409 like high salinities would render it more light sensitive (see paragraph 4.2.). This general
410 response was likely related to the linear lowering of NPQ with high salinities together with the
411 anti-oxidative stress response (i.e. strong DT synthesis). It supports the obvious salinity (and
412 light) sensitivity of *P. vanheurckii*. This is confirmed by previous studies on another
413 tychoplankton representative (*Cylindrotheca closterium*) (Rijstenbil, 2003, 2005; Roncarati et
414 al., 2008). In response to the high salinity- and/or HL-dependent ROS generation, the
415 intracellular pools and activity of important players of the oxidative stress response (i.e. the
416 reduced glutathione-GSH, the superoxide dismutase-SOD enzyme) increased. Albeit such
417 protective response, cells could not avoid significant lipid peroxidation (Roncarati et al.,
418 2008). Peroxidation of lipids of the thylakoid membrane disturbs osmoregulation (Rijstenbil,
419 2003, 2005) which might explain the synthesis of intracellular osmoregulators like free sugars
420 (mannose, Paul, 1979), amino acids (taurine, Jackson et al., 1992; and proline, Natana

Murugaraj and Jeyachandran, 2007). Moreover, leakage of the thylakoid membrane can impair the build-up of the transthylakoid ΔpH (i.e. loss of membrane potential, Rijstenbil, 2005), which would well explain the NPQ decrease with increasing salinities (Lavaud and Lepetit, 2013; Lavaud and Goss, 2014). All together, these observations fit well with an adaptation of tychoplankton to salinities ~33 as it is mostly the case in the water column (when cells are resuspended at high tide) or buried in sediment (when cells settle down at low tide) as observed here from -0.5 cm down (see paragraph 4.1.).

4.3.3. Epipsammon

The response of epipsammon to salinity changes is much less documented. To our knowledge, only Scholz and Liebezeit (2012) investigated the negative impact of salinity on the growth of episammic species like *Achnantes* spp. and *Amphora* spp.. Because it lives attached to sediment particles and the light penetration is deeper in (less cohesive) sandy sediment, the epipsammon photophysiological response to HL is efficient (Barnett et al., 2014). Here, under LL, *B. lucens* was relatively insensitive to high salinity. Nevertheless, and although DES and NPQ were high, the ability to decrease the excitation pressure on PSII during HL exposure was partially abolished by salinities > 37-41. As a consequence, $rETR_m$ decreased, thus potentially impairing the photosynthetic productivity. While *B. lucens* is well adapted to cope with HL and with high salinity, it appears less well adapted to the combination of the two. This fits well with the fact that in its natural habitat, even if the light climate can be extreme, changes in salinity remain moderate i) even in the first 0.5 cm of sediment, and ii) especially deeper where a significant part of the epipsammon biomass inhabits, as shown here (see paragraph 4.1.).

4.4. Conclusions

The photophysiology of representatives of the three main groups of intertidal MPB diatoms (i.e. epipelon, epipsammon and tychoplankton) differentially responded to a high salinity stress alone or combined with moderate HL exposure. While the representative of epipelon was relatively insensitive to these conditions, the tychoplankton representative was highly sensitive to both, and the epipsammon representative was sensitive mainly to the stress combination. These specific responses fitted well with i) their natural habitat (i.e. more or less cohesive sediment) for which light climate and changes in salinity differ, ii) their growth form (i.e. motile, immotile or amphibious) which determines their probability to be confronted to a combined high salinity-HL stress, and their capacity to eventually escape from it (i.e. epipelon). Although light and temperature are regarded as major drivers of the photosynthetic productivity of MPB in Western Europe intertidal mudflats (Kromkamp et al., 2006), salinity increase during emersion obviously can non-negligibly modulate the MPB photosynthesis when it is combined with HL (and temperature) according to the weather conditions and sediment type. It nevertheless appears mostly restricted to epipsammon and tychoplankton, and in field conditions, although likely stronger than in the present study, its effect probably remains negligible compared to HL stress.

Acknowledgements

The authors acknowledge the Centre National de la Recherche Scientifique-CNRS (program ‘chercheurs invités’ 2011), the University of La Rochelle-ULR (ACI ‘chercheurs invités’ 2011), the Region Poitou-Charentes (program ‘chercheurs invités’ 2013), the Contrat Plant Etat Région-CPER Littoral (2007-13), and the Natural Science and Engineering Research Council of Canada-NSERC (grant #262210-2011) for their financial support.

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685 **Figure legends**

686

687 **Figure 1**

688 **Evolution of the pore-water sediment salinity (A, B) and chlorophyll *a* (Chl *a*) biomass**
689 **(C) during emersion in the upper sediment layer (0-0.5 cm, black columns / 0.5-1 cm,**
690 **white columns for A- and B-; Mud, black columns / Muddy sand, white columns for C-)**
691 **in two sites of the French Atlantic coast with two different sediment types (A- Mud; B-**
692 **Muddy sand) in Spring.** The representative day was 2012/04/20 for the muddy site and
693 2012/05/06 for the muddy sandy site. They showed the following features: emersion maximum
694 at 11:25 AM ± 5 min, no rain, sediment surface temperature = 16.6 ± 1.8 °C and 20.6 ± 4.3 °C
695 for the muddy and the muddy sandy sites, respectively; based on these temperatures, a 20°C
696 experimental temperature was further used. Values are averages ± standard deviation (n = 3).

697

698 **Figure 2**

699 **Photophysiological parameters in *Navicula phyllepta*, *Biremis lucens* and**
700 ***Plagiogrammopsis vanheurckii* exposed to different salinities (33 to 45)**. Abbreviations: LL,
701 growth low light (60 µmol m^{-2} s^{-1} photons); HL, after 1h high light (600 µmol m^{-2} s^{-1} photons)
702 treatment; α-Alpha, maximum light efficiency use; $rETR_m$, maximum relative electron
703 transport rate; E_k, light saturation coefficient. Values are averages ± standard deviation (n = 3).

704

705 **Figure 3**

706 **Rate of de-epoxidation (DES) of diadinoxanthin (DD) to diatoxanthin (DT) in *Navicula***
707 ***phyllepta*, *Biremis lucens* and *Plagiogrammopsis vanheurckii* exposed to different salinities**

708 **(33 to 45).** Abbreviations: DES = [(DD + DT) / DT x 100]; LL, growth low light (60 µmol m^{-2}
709 s^{-1} photons), white columns; HL, after 1h high light (600 µmol m^{-2} s^{-1} photons) treatment,
710 black columns. Values are averages ± standard deviation (n = 3).
711

712 **Table 1_Juneau et al.**

713 **Pore-water salinity measured during emersion in the upper sediment layer (first 1 cm) in**
714 **two sites of the French Atlantic coast with two different sediment types and at different**
715 **seasons.** Values are averages ± standard deviation (n = 9 to 12).

716

Sediment type	Season period	Min	Max	Emersion Δ max	Emersion Δ mean
Mud (95.1 ± 0.1 % mud/ 4.9 ± 0.1% sand)	Winter 02/19-02/24	29.0 ± 1.2	34.1 ± 1.1	2.3	1.3 ± 0.6
	Spring 04/19-04/22	32.5 ± 1.1	38.8 ± 1.1	5.1	4.4 ± 1.0
	Summer 07/13-07/26	35.8 ± 0.2	48.2 ± 0.7	8.3	4.6 ± 2.7
Muddy sand (57.9 ± 7.9% sand/ 42.1 ± 7.9% mud)	Spring 04/05-07/05	30.8 ± 1.0	35.4 ± 2.8	3.2	1.9 ± 1.2
	Fall 09/30-10/02	32.8 ± 0.4	37.3 ± 2.7	3.8	3.7 ± 0.2

717

Table 2_Juneau et al.

Photophysiological parameters used in this study, their meaning and how they were measured. Abbreviations: Chl, chlorophyll; DD, diadinoxanthin; DT, diatoxanthin; E, light intensity; PSII, photosystem II; RLCs, Rapid Light Curves. See the Materials and Methods section for further details.

Parameter	Unit	Definition	Photophysiological meaning	Measurement conditions
F_0	No units	Minimum PSII Chl fluorescence yield	Used to calculate F_v/F_m (see below)	Measured with RLCs after 15 min of dark acclimation
F_m	No units	Maximum PSII Chl fluorescence yield	Used to calculate F_v/F_m and NPQ (see below)	Measured with RLCs during a saturating pulse after 15 min of dark acclimation
F_v/F_m	No units	Maximum PSII quantum yield; $F_v/F_m = (F_m - F_0) / F_m$	Maximum potential quantum efficiency of PSII photochemistry	See the above measurement conditions for F_0 and F_m

F_m'	No units	F_m for illuminated cells	Used to measure NPQ and rETR	Measured with RLCs during a saturating pulse after 60 s of illumination at specific E
ΦPSII	No units	Operational PSII quantum yield; ΦPSII = (F_m' - F) / F_m'	Maximum effective quantum efficiency of PSII photochemistry	See the above measurement conditions for F_0 and F_m; F is the steady-state of Chl fluorescence measured after 60 s illumination at specific E
NPQ	No units	Non-photochemical quenching of Chl fluorescence; NPQ = F_m / F_m' - 1	Estimates the photoprotective dissipation of excess energy	Measured with RLCs
rETR	µmol electrons m^{-2} s^{-1}	Relative electron transport rate of PSII;	Effective quantum yield of photochemistry *vs*. E	Measured with RLCs

		rETR = ΦPSII x E		
α	µmol electrons m^{-2} s^{-1} / µmol photons. m^{-2}. s^{-1}	rETR-E curve initial slope	Maximum light efficiency use	Derived from fitted rETR-E curves (Eilers and Peeters, 1988)
$rETR_m$	µmol electrons m^{-2} s^{-1}	rETR-E curve asymptote	Maximum relative photosynthetic electron transport rate	Derived from fitted rETR-E curves (Eilers and Peeters, 1988)
E_k	µmol photons. m^{-2}. s^{-1}	$E_k = rETR_m / \alpha$	Light saturation coefficient	Derived from fitted rETR-E curves (Eilers and Peeters, 1988)
NPQ_m	No units	Maximum NPQ	Maximum ability for dissipation of excess energy	Measured at maximum E of RLCs
DES	%	DES = [DT / (DD+DT)	De-epoxidation state of DD to DT	Measured during growth at LL

		x 100]		and after 1 h HL treatment

Table 3_Juneau et al.

Photochemical potential and non-photochemical fluorescence quenching in *Navicula phyllepta* (N.p.), *Biremis lucens* (B.l.) and *Plagiogrammopsis vanheurckii* (P.v.) exposed to different salinities. Abbreviations: LL, growth low light (60 µmol m^{-2} s^{-1} photons); HL, high light (600 µmol m^{-2} s^{-1} photons). Definitions and conditions of measurement of F_v/F_m, ΦPSII and NPQ_m are listed in Table 2. Values are averages ± standard deviation (n = 3).

		LL			HL		
Species	Salinity	F_v/F_m	ΦPSII	NPQ_m	F_v/F_m	ΦPSII	NPQ_m
N.p.	33	0.724 ± 0.011	0.599 ± 0.030	0.306 ± 0.060	0.667 ± 0.028	0.551 ± 0.031	0.547 ± 0.053
	37	0.727 ± 0.019	0.597 ± 0.031	0.315 ± 0.044	0.659 ± 0.007	0.568 ± 0.041	0.485 ± 0.070
	41	0.730 ± 0.010	0.600 ± 0.068	0.250 ± 0.022	0.662 ± 0.006	0.559 ± 0.038	0.542 ± 0.017
	45	0.724 ± 0.015	0.600 ± 0.051	0.259 ± 0.038	0.674 ± 0.009	0.571 ± 0.019	0.653 ± 0.016
B.l.	33	0.694 ± 0.010	0.563 ± 0.009	0.323 ± 0.097	0.629 ± 0.009	0.504 ± 0.003	0.588 ± 0.142
	37	0.694 ± 0.012	0.554 ± 0.016	0.315 ± 0.071	0.602 ± 0.010	0.498± 0.040	0.864 ± 0.186
	41	0.689	0.569	0.332	0.607	0.496	0.736

		± 0.020	± 0.008	± 0.024	± 0.041	± 0.039	± 0.332
	45	0.703 ± 0.010	0.560 ± 0.019	0.357 ± 0.103	0.627 ± 0.019	0.530 ± 0.038	0.782 ± 0.271
P.v.	33	0.588 ± 0.034	0.312 ± 0.024	0.791 ± 0.141	0.370 ± 0.032	0.265 ± 0.031	1.003 ± 0.048
	37	0.539 ± 0.009	0.292 ± 0.095	0.622 ± 0.129	0.352 ± 0.028	0.270 ± 0.021	1.178 ± 0.103
	41	0.555 ± 0.021	0.291 ± 0.017	0.625 ± 0.011	0.336 ± 0.051	0.213 ± 0.032	0.908 ± 0.163
	45	0.577 ± 0.055	0.316 ± 0.041	0.542 ± 0.019	0.359 ± 0.018	0.221 ± 0.066	0.870 ± 0.143

Table 4_Juneau et al.

Pigments in *Navicula phyllepta* (N.p.), *Biremis lucens* (B.l.) and *Plagiogrammopsis vanheurckii* (P.v.) exposed to different salinities. Abbreviations: LL, growth low light (60 µmol m^{-2} s^{-1} photons); HL, high light (600 µmol m^{-2} s^{-1} photons). Chl *a*, chlorophyll *a*; Chl *c*, chlorophyll *c*; Fx, fucoxanthin; DD, diadinoxanthin; DT, diatoxanthin; ß-car, ß-carotene. Chl *a* is in pg. $cell^{-1}$; other pigments are in mol. 100 mol Chl a^{-1}. Values are averages ± standard deviation (n = 3).

		LL						HL					
Species	Salinity	Chl *a*	Chl *c*	Fx	ß-car	DD	DT	Chl *a*	Chl *c*	Fx	ß-car	DD	DT
N.p.	33	0.830 ± 0.020	33.2 ± 0.9	151.0 ± 0.9	8.6 ± 8.3	33.7 ± 2.3	3.5 ± 0.2	0.716 ± 0.019	37.1 ± 3.1	134.2 ± 3.5	13.0 ± 5.2	30.8 ± 1.0	9.1 ± 1.9
	37	0.821 ± 0.065	42.2 ± 8.9	157.0 ± 11.4	8.3 ± 8.5	34.1 ± 1.1	3.1 ± 0.1	0.621 ± 0.043	35.9 ± 2.8	131.8 ± 1.7	13.9 ± 3.7	29.1 ± 2.9	9.9 ± 1.8
	41	0.812 ± 0.016	42.7 ± 0.8	152.1 ± 5.8	14.0 ± 6.4	33.8 ± 5.6	3.0 ± 0.4	0.652 ± 0.053	34.8 ± 2.4	127.8 ± 1.7	12.2 ± 6.7	29.6 ± 1.6	9.4 ± 2.6
	45	0.793 ± 0.040	40.9 ± 1.9	150.1 ± 6.1	14.1 ± 5.8	33.2 ± 5.0	3.0 ± 0.2	0.607 ± 0.048	33.2 ± 0.6	127.5 ± 1.4	11.3 ± 5.3	28.8 ± 0.2	11.8 ± 2.6

B.l.	33	1.830 ± 0.280	27.1 ± 2.7	76.8 ± 4.5	7.1 ± 6.2	19.5 ± 0.2	1.7 ± 0.8	1.642 ± 0.201	25.7 ± 1.2	72.5 ± 4.4	2.2 ± 1.9	14.5 ± 2.1	8.6 ± 0.2
	37	1.661 ± 0.105	24.3 ± 3.2	70.0 ± 6.6	2.8 ± 1.4	17.7 ± 0.9	1.2 ± 1.0	1.626 ± 0.186	24.4 ± 2.9	69.2 ± 8.9	3.5 ± 0.5	13.8 ± 2.1	9.7 ± 0.8
	41	1.883 ± 0.210	29.0 ± 4.6	87.3 ± 7.3	3.6 ± 0.5	23.4 ± 0.8	1.1 ± 0.9	1.554 ± 0.088	22.8 ± 0.9	63.9 ± 3.5	3.5 ± 0.1	12.8 ± 2.2	8.9 ± 1.1
	45	1.749 ± 0.220	26.6 ± 2.8	76.7 ± 7.9	4.1 ± 1.2	18.9 ± 0.3	1.1 ± 1.3	1.731 ± 0.157	25.5 ± 0.9	72.7 ± 2.7	3.7 ± 0.6	15.7 ± 1.7	8.8 ± 0.1
P.v.	33	2.010 ± 0.410	29.6 ± 4.4	97.8 ± 12.2	1.9 ± 0.0	13.5 ± 2.2	7.3 ± 2.3	2.070 ± 0.419	30.8 ± 3.2	97.9 ± 3.9	1.9 ± 0.3	9.9 ± 0.9	13.4 ± 1.4
	37	2.366 ± 0.441	30.9 ± 0.9	103.5 ± 6.4	2.1 ± 0.3	14.5 ± 0.0	7.8 ± 2.1	1.679 ± 0.503	27.1 ± 2.7	90.9 ± 9.5	2.2 ± 0.1	8.8 ± 1.6	11.4 ± 3.0
	41	2.034 ± 0.488	25.6 ± 2.4	86.2 ± 4.7	1.4 ± 0.1	11.8 ± 0.4	6.2 ± 1.7	1.586 ± 0.508	26.8 ± 1.7	89.7 ± 2.3	1.9 ± 0.3	8.5 ± 0.4	11.9 ± 0.1
	45	2.095	26.8	88.3	3.4	11.0	8.6	1.456	23.9	78.4	2.2	7.4	10.7

		± 0.388	± 5.1	± 11.1	± 2.1	± 4.7	± 0.5	± 0.371	± 5.1	± 12.8	± 0.5	± 1.0	± 1.3

Table 5_Juneau et al.

Results of the 2 factor ANOVA procedure for the comparison of the parameters measured in *Navicula phyllepta* (N.p.), *Biremis lucens* (B.l.) and *Plagiogrammopsis vanheurckii* (P.v.) exposed to different salinities and lights. Code: white + n.s. (non significant): $p > 0.05$; light grey + *: $p < 0.05$; medium grey + **: $p < 0.01$; dark grey + ***: $p < 0.001$; arrow up, increase of values; arrow down, decrease of values. The two factor ANOVA analysis was performed on data shown in Tables 3 and 4, Fig. 2 and 3.

Salinity	N.p.	B.l.	P.v.
F_v/F_m	n.s.	n.s.	n.s.
ΦPSII	n.s.	n.s.	n.s.
α	n.s.	n.s.	* ↓
$rETR_m$	n.s.	* ↓	*** ↑
E_k	n.s.	* ↓	** ↑
NPQ_m	n.s.	n.s.	* ↓
DES	n.s.	n.s.	n.s.
DD	n.s.	n.s.	n.s.
DT	n.s.	n.s.	n.s.
Chl *a*	n.s.	n.s.	n.s.
Chl *c*	n.s.	n.s.	n.s.
Fx	n.s.	n.s.	n.s.
ß-car	n.s.	n.s.	n.s.

Light	N.p.	B.l.	P.v.
F_v/F_m	*** ↓	*** ↓	*** ↓
ΦPSII	* ↓	*** ↓	* ↓
α	n.s.	*** ↓	** ↓
$rETR_m$	n.s.	n.s.	n.s.
E_k	n.s.	** ↑	* ↑
NPQ_m	*** ↑	*** ↑	*** ↑
DES	*** ↑	*** ↑	*** ↑
DD	n.s.	*** ↓	*** ↓
DT	*** ↑	** ↑	*** ↑
Chl *a*	*** ↓	n.s.	n.s.
Chl *c*	n.s.	n.s.	n.s.
Fx	n.s.	n.s.	n.s.
ß-car	n.s.	n.s.	n.s.

Light x Salinity	N.p.	B.l.	P.v.
F_v/F_m	n.s.	n.s.	n.s.
ΦPSII	n.s.	n.s.	n.s.
α	n.s.	n.s.	n.s.
$rETR_m$	n.s.	n.s.	n.s.
E_k	n.s.	*	n.s.
NPQ_m	**	n.s.	n.s.
DES	n.s.	n.s.	n.s.
DD	n.s.	n.s.	n.s.
DT	n.s.	n.s.	n.s.
Chl *a*	n.s.	n.s.	n.s.
Chl *c*	n.s.	n.s.	n.s.
Fx	n.s.	n.s.	n.s.
ß-car	n.s.	n.s.	n.s.

Figure 1_Juneau et al.

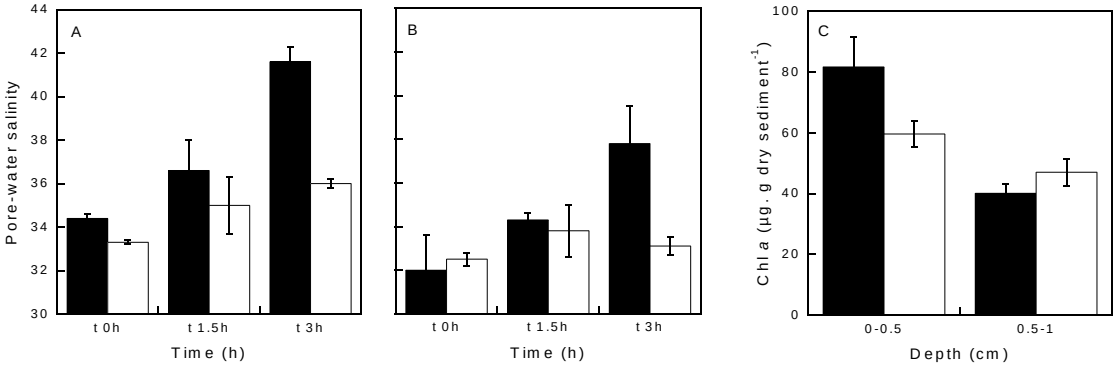

Figure 2_Juneau et al.

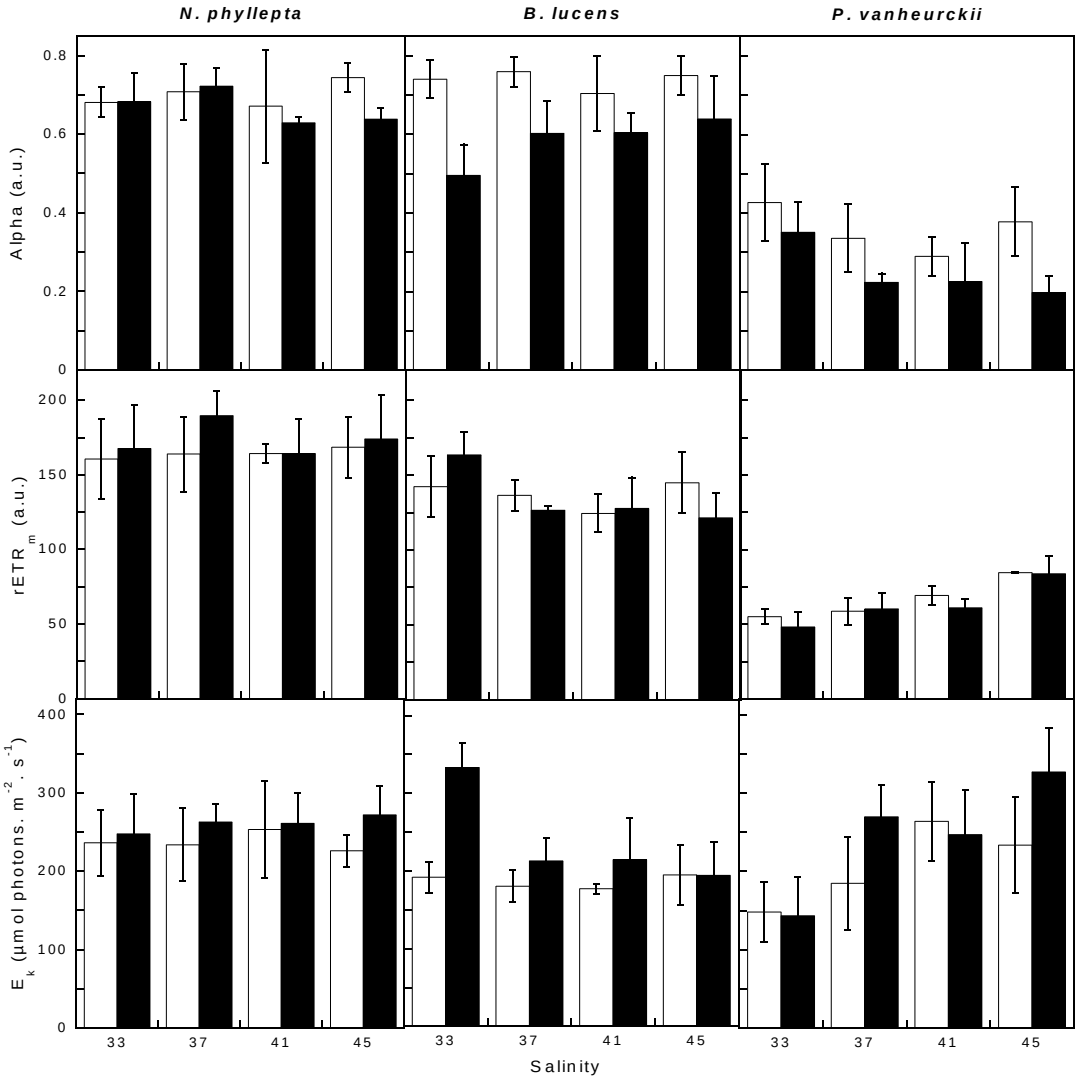

Figure 3_Juneau et al.